Full Length Research Paper

Antimicrobial and antioxidant evaluation of various parts of *Cola milleni* K. Schum plant

Orisakeye, O. T. and Ojo, A. A.

Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

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The methanol extracts of the various plant parts were screened for anti-oxidant activity by thin layer chromatography using 2,2-diphenyl-1-dipicrylhydrazyl (DPPH) while their in vitro antimicrobial evaluation were determined by agar diffusion method and bioautographic technique using *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as test organisms; aqueous solvent served as control. The result showed that methanol extracts of the epicarp were found to be stronger against the entire test organisms except *E. coli* and were also found to possess strong antioxidant activity. The seed from the fruit, stem and root bark of the plant were not active against the entire test organisms. The antimicrobial property shown by the leaf is an evidence of the ethnomedicinal uses of the plant. Phytochemical screening of the plant showed the presence of saponins, peptides and sugars.

Key words: *Cola milleni*, 2,2-diphenyl-1-dipicrylhydrazyl (DPPH), *Escherichia coli*, *Bacillus subtilis*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, antimicrobial, antioxidant activity.

INTRODUCTION

*Cola milleni* K. Schum is one of 125 species from the genus *Cola* Schott & Endl from the family Sterculiaceae (Ratsch, 2005). Sterculiaceae is a botanical name for a group of flowering plants at the rank of family, as is true for any botanical name, the circumscription, status and placement for the taxon varies with taxonomic point of view. The family name is based on the genus *Sterculia*. As traditionally circumscribed, the sterculiaeae, Malvaceae, Bombacaceae, and Tiliaceae comprise the “core Malvales” of the croquist system and the close relationship among these families are generally recognised (Watson and Dallwitz, 1992). The genus was formerly classified in the family Malvaceae subfamily Sterculioideae and was later transferred into the separate family Sterculiaceae (Sonibare, 2009). *Cola* is one of the most popular genera in the family of about 70 genera, totaling around 1,500 species of tropical trees and shrubs. It is related to the genus *Theobroma* which is also part of the family (Orisakeye and Olugbade, 2012).

*Cola milleni* K. Schum is a tree that grows to 12 m high, occasionally to 20 m with a low crown of arching branches; deciduous, of closed and transition forest, tending toward the drier parts, in Ivory Coast to Southern Nigeria (Adegoke et al., 1968). As a tree, it grows vigorously, and it has been successfully called monkey kola in English and Atewo-edun as Yoruba people would call it or achi okokoro (Ibo).

The bark has been reported to show alkaloids (Adegoke et al., 1968). Odugbemi (2006) reported that leaves of *C. milleni* are used in the treatment of ringworm,
ringworm, scabies, gonorrhoea, dysentery and ophthalmic. The fruit is bright red in a stellate cluster; its seed is covered with a felled fibrous coal. The kernel is edible. The wood is white and very resilient. It is used in Nigeria for the stock of the cross bow and in Liberia for rat traps and bows.

The antimicrobial property and its phytochemistry of the leaf ethanol extract of C. milleni was also done using human strain (Sonibare et al., 2009). According to Adeniyi et al. (2004), only the leaf and stem bark of C. milleni were reported not to be active at the highest concentration of 1000 microg/ml. Only the methanol extract root bark of C. milleni was found to be potent against both Mycobacterium bovis and strains of Mycobacterium vaccae. In spite of the popularity of the plant in traditional application previous phytochemical and antimicrobial studies have been limited to the leaf and stem bark. The present study examines the seed, epicarp, leaf, stem and root bark for their antioxidant and antimicrobial property.

MATERIALS AND METHODS

Plant

All the plant parts materials were collected from a tree located on Obafemi Awolowo University (OAU) campus (Road 1). It was authenticated by comparison with herbarium specimens by the Department of botany, OAU.

Extraction of plant material

The fruits of C. milleni were separated into epicarp and seeds. The epicarp was then macerated (930 g) in methanol (100%) at room temperature for 72 h. The seed (1630 g), stem bark (1107 g), leaf (463 g) and root bark (450 g) were air-dried, powdered and extracted in aqueous methanol at room temperature for 48 h. The mixture of each extraction was then filtered using filter paper. The filtrate was concentrated to dryness in vacuo to yield crude extract C. milleni stem bark (CMSB) (23.06 g), C. milleni root bark (CMRB) (24.28 g), C. milleni leaf (CML) (31.13 g), C. milleni epicarp (CME) (12.13 g) and C. milleni seed (CMS) (15.34 g).

Antioxidant screening

Crude extracts of all the parts of the plant were screened for antioxidant activity. The brief description of the procedure is as follows: A solution of the test material in method was spotted on the thin layer chromatographic plate and developed using a suitable solvent system. This was sprayed with DPPH reagent. The chromatogram was exposed to daylight until the purple violet background was bleached. Only zones where the colour turned yellow within the first fifteen minutes after spraying were recorded as positive results (that is, possess antioxidant activity). The result of the screening of the different plant materials are summarized in Table 1.

Antimicrobial screening

The agar diffusion (cup plate) method was used for this examination. Molten and cooled agar 60 ml (45°) were separately inoculated with the nutrient broth culture of the test organisms (0.6 ml) and mixed thoroughly. The inoculated medium was then carefully poured into sterile petri dishes (24 cm petri dish) and allowed to set. Thereafter, cups (8 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions 100 ul each were then introduced into each of the cups ensuring that no spillage occurred. Also, the same volume of the standard antimicrobial agent and the solvent were introduced into some of the cups to act as positive and negative controls, respectively. The plates were left at room temperature for 2 h to allow for diffusion into the medium and thereafter incubated face upwards at 37°C for 24 h. Sample was tested in duplicate and diameters of zone of inhibition were measured to the nearest millimetre using transparent ruler (Onawumi, 1997).

Bioautography technique for anti-microbial screening

The method involved an overlay of inoculated agar medium on developed silica gel thin layerer chromatography (TLC) glass plate followed by incubation at 37°C for 24 h. Zones of inhibition were detected as clear white areas over a purple background (Onawumi, 1997).

Phytochemical screening

The dried, pulverised leaves were subjected to phytochemical analysis to screen for peptides, cyclopeptides and sugars.

Test for peptides

The extracts were examined for presence of peptides using TLC technique with solvent system dichloromethane: methanol: acetic acid (8:2:2) as the mobile phase. A reverse phase plate was the stationary phase while freshly prepared ninhydrin in acetone was the detecting agent. Presence of cyclopeptides was studied in the crude extract by developing two spotted silica plates (A and B) in solvent system dichloromethane: methanol: acetic acid (8:2:2). Sample on one of the plates (B) was hydrolyzed by heating the plate in a covered glass vessel containing concentrated hydrochloric acid at 100°C for 1 h. Both plates (A and B) were subsequently sprayed with ninhydrin in acetone. Any ninhydrin positive spot on B but absent in A is taken as positive for the presence of cyclopeptides.

Test for sugars

A solution of the test material was spotted on the TLC plate along side with glucose, sucrose, fructose, lactose and developed using a suitable solvent system. This was sprayed with p-anisaldehyde-sulphuric acid.

Test for phenolics using ferric chloride

It is used in the detection of phenolics. A solution of FeCl₃ (1 g) in methanol (40 ml) is prepared for spraying on TLC plates.

RESULTS

The results of antioxidant tests and antimicrobial activities
Table 1. Antimicrobial activities of both the crude extract from Sterculia tragacantha and Cola milleni in 80mg/ml concentration in methanol:H₂O (1:1).

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Diameter of zone of inhibition (mm)</th>
<th>STRB</th>
<th>CML</th>
<th>CME</th>
<th>CMS</th>
<th>STSB</th>
<th>CMRB</th>
<th>CMSB</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli NCTC 10418</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus NCTC 6571</td>
<td>5</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 10145</td>
<td>6.5</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis NCTC 8236</td>
<td>6</td>
<td>6.5</td>
<td>10</td>
<td>-</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Cup size = 8.0 mm, - = no activity.

Table 2. Antioxidant activities of the crude extracts of various parts of Cola milleni.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Source</th>
<th>Time taken for colour development (DPPH method)</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML</td>
<td>Leaf</td>
<td>No reaction</td>
<td>-</td>
</tr>
<tr>
<td>CMS</td>
<td>Seed</td>
<td>Immediate</td>
<td>Strong</td>
</tr>
<tr>
<td>CMRB</td>
<td>Root bark</td>
<td>10 min</td>
<td>Weak</td>
</tr>
<tr>
<td>CMSB</td>
<td>Stem bark</td>
<td>10 min</td>
<td>Weak</td>
</tr>
<tr>
<td>CME</td>
<td>Epicarp</td>
<td>5 min</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Peptides

All the various parts of the plant was screened for peptides. Only C. milleni epicarp showed the presence of peptides.

Cyclopeptides

Out of all the various parts of the plant screened for cyclopeptides, C. milleni stem bark showed the presence of cyclopeptides. Unlike the case of peptides, cyclopeptides are detectable with ninhydrin only after hydrolyses, since the detection involve reaction of ninhydrin with the free primary amino function. However, amides are expected to show similar characteristics as the cyclopeptides in the test system.

Sugars

C. milleni epicarp showed the presence of fructose and C. milleni seed showed the presence of both the lactose and fructose. This would explain why the mucilagenous cover of the seed is sweet. On the other hand, no sugars were detected in the remaining parts of the plant.

Phenolics

C. milleni root bark and epicarp showed the presence of phenols. On the other hand, no phenols were detected in the remaining parts of C. milleni examined.

DISCUSSION

The crude extracts of the various parts of C. milleni showed reaction with DPPH after some minutes except the leaf. The CMS showed immediate colour reaction and it was a strong anti-oxidant activity. The stem and root barks showed a colour reaction after 10 min and it was a weak anti-oxidant activity. This is suggestive that further work could be done on it to know the active principle that is responsible for the activity. The CME possess strongly anti-oxidant activity after 5 min. This is also suggestive that if further work is done on it extensively the seed and the epicarp of the plant could serve as a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate, vitamin C and vitamin E succinate combined, and beta carotene.

In this study, various parts of the crude extracts of C. milleni were subjected to preliminary antimicrobial test. C. Milleni leaves were active against gram-negative Pseudomonas aeruginosa at a minimum diameter of zone of inhibition of 6.5 mm and gram-positive Bacillus subtilis at a minimum diameter of zone of inhibition of 6.5 mm but not active against Escherichia coli and Staphylococcus aureus. Interestingly, the antimicrobial activity of the leaf was line with the previous antimicrobial works of C. milleni (Sonibare et al., 2009; Adeniyi et al., 2004; Ebana et al., 199; Reid et al., 2005).

CME showed no activity with E. coli but were active against S. aureus at a minimum zone of inhibition of 15 mm, P. aeruginosa at a minimum zone of inhibition of 6 mm, B. subtilis at minimum zone of inhibition of 10 mm.
CMS, CMRB and CMSB did not show any activity with any of the test organisms. Hopefully, CMS, CMRB and CMSB are not wastes materials because of their antioxidant activities. Further work is being done by the author of this paper to isolate the active compounds responsible for both the antimicrobial and antioxidant activity each.

Conclusion

The antimicrobial property of the leaf justifies its use in treating ringworm, scabies, dysentery, gonorrhoea and opthalmic. Unfortunately, people lick the seed and throw away its epicarp. This research findings has shown that the epicarp possesses strong antimicrobial activity and also show a strong antioxidant activity implying that it could also be used to treat ringworm, scabies, dysentery to mention a few and could serve as a free radical scavenger.

REFERENCES


